

DOCKET NO.: TIBO-0008 (TIP0017USA)
Application No.: 09/640,787

PATENT

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Currently amended) A method for detection of mutations in the pol gene of HIV-1 isolates comprising the steps of:
 - a) isolation of a sample comprising HIV-1 RNA,
 - b) PCR amplifying RNA from said sample using ~~at least~~ a primer chosen from ~~the~~ outer primer ~~groups~~ as represented in SEQ ID No: 1 ~~or~~ and SEQ ID No: 2, to obtain a primary PCR product,
 - c) PCR amplifying said primary PCR products using at least a 5' and 3' primer chosen from the inner primer group SEQ ID No: 3, SEQ ID No: 4, SEQ ID NO: 5, and SEQ ID No: 6, to obtain a secondary PCR product, and
 - d) sequencing said secondary PCR product.
2. (Previously presented) A method according to Claim 1, wherein said secondary PCR product is sequenced using at least one sequencing primer chosen from the group SEQ ID No: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, and SEQ ID No: 12.
3. (Original) A method according to Claim 1, wherein said RNA is viron RNA extracted from said sample.
4. (Previously presented) A method according to Claim 1, wherein said secondary PCR product is sequenced using at least one sequencing primer chosen from the group SEQ ID NO: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, and SEQ ID No: 12; and
wherein at least one of said sequencing primer is replaced by one or a pair of replacement primers, wherein said one or a pair of replacement primers obtain sequence from the region that said at least one sequencing primer is expected to cover.
5. (Previously presented) A method according to Claim 1, wherein said secondary PCR product is sequenced using at least one sequencing primer chosen from primers up to 1, 2, 3, or 4 nucleotides upstream or downstream primer regions chosen from

DOCKET NO.: TIBO-0008 (TIP0017USA)
Application No.: 09/640,787

PATENT

the group SEQ ID No: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, and SEQ ID No: 12.

6. (Previously presented) A method according to Claim 1, wherein the outer primer is chosen from primers up to 1, 2, 3, or 4 nucleotides upstream or downstream primer regions chosen from the group SEQ ID No: 1 and SEQ ID No: 2.

7. (Previously presented) A method according to Claim 1, wherein the inner primer is chosen from primers up to 1, 2, 3, or 4 nucleotides upstream or downstream primers regions chosen from the group SEQ ID No: 3, SEQ ID No: 4, SEQ ID No: 5, and SEQ ID No: 6.

8. (Original) A method according to Claims 1, wherein the sample contains free viron particles or virus infected cells.

9. (Previously presented) A method according to Claim 1, wherein said primary PCR product is sequenced using at least one sequencing primer chosen from the group SEQ ID No: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, and SEQ ID No: 12.

10-20 (Canceled)

21. (Previously presented) A method for detection of mutations in the pol gene of HIV-1 isolates comprising the steps of:

- a) isolation of a sample comprising HIV-1 RNA,
- b) PCR amplifying RNA from said sample using an outer primer with SEQ ID No: 1 and SEQ ID No: 2 to obtain a primary PCR product,
- c) PCR amplifying said primary PCR products using a 5' and 3' primer chosen from an inner primer from the group SEQ ID No: 3, SEQ ID No: 4, SEQ ID NO: 5, and SEQ ID No: 6, to obtain a secondary PCR product, and
- d) sequencing said secondary PCR product.

22. (Previously presented) A method according to Claim 21, wherein said secondary PCR product is sequenced using at least one sequencing primer chosen from SEQ ID No: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, and SEQ ID No: 12.

23. (Previously presented) A method according to Claim 21, wherein said RNA is viron RNA extracted from said sample.

DOCKET NO.: TIBO-0008 (TIP0017USA)
Application No.: 09/640,787

PATENT

24. (Previously presented) A method according to Claim 21, wherein said secondary PCR product is sequenced using at least one sequencing primer chosen from SEQ ID NO: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, and SEQ ID No: 12; and

wherein at least one of said sequencing primer is replaced by one or a pair of replacement primers, wherein at least one of said replacement primers is at least one from the group SEQ ID No: 13 and SEQ ID No: 14 for sequencing primer SEQ ID No: 7, SEQ ID No: 15 and SEQ ID No: 16 for sequencing primer SEQ ID No: 8, SEQ ID No: 16 and SEQ ID No: 17 for sequencing primer SEQ ID NO: 9, SEQ ID No: 4 and SEQ ID No: 18 for sequencing primer SEQ ID NO: 10, SEQ ID No: 18 and SEQ ID No: 19 for sequencing primer SEQ ID NO: 11, and SEQ ID No: 20 and SEQ ID No: 21 for sequencing primer SEQ ID NO: 12.

25. (Previously presented) A method according to Claim 21, wherein said secondary PCR product is sequenced using at least one sequencing primer chosen from primers up to 1, 2, 3, or 4 nucleotides upstream or downstream primer regions chosen from at least one of SEQ ID No: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, and SEQ ID No: 12.

26. (Previously presented) A method according to Claim 21, wherein the outer primer is chosen from primers up to 1, 2, 3, or 4 nucleotides upstream or downstream primer region with SEQ ID No: 1 and SEQ ID No: 2.

27. (Previously presented) A method according to Claim 21, wherein the inner primer is chosen from primers up to 1, 2, 3, or 4 nucleotides upstream or downstream primer region with SEQ ID No: 3, SEQ ID No: 4, SEQ ID No: 5, and SEQ ID No: 6.

28. (Previously presented) A method according to Claims 21, wherein the sample contains free viron particles or virus infected cells.

29. (Previously presented) A method according to Claim 21, wherein said primary PCR product is sequenced using at least one sequencing primer chosen from the group SEQ ID No: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10, SEQ ID No: 11, and SEQ ID No: 12.

30. (Previously presented) A method according to Claim 21, wherein said inner primer has SEQ ID No: 3, SEQ ID No: 4, SEQ ID NO: 5, and SEQ ID No: 6.

DOCKET NO.: TIBO-0008 (TIP0017USA)
Application No.: 09/640,787

PATENT

31. (Previously presented) A method according to Claim 30, wherein said outer primer is chosen from primers up to 1, 2, 3, or 4 nucleotides upstream or downstream primer region with SEQ ID No: 1 and SEQ ID No: 2.

32. (Previously presented) A method according to Claim 30, wherein said inner primer is Chosen from primers up to 1, 2, 3, or 4 nucleotides upstream or downstream primer region with SEQ ID No: 3, SEQ ID No: 4, SEQ ID NO: 5, and SEQ ID No: 6.

33. (Previously presented) A method according to claim 30, wherein said RNA is viron RNA extracted from said sample.

34. (Previously presented) A method according to claim 30, wherein said sample contains free viron particles or virus infected cells.